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Urine and Serum Cathepsin B Concentrations in the Transitional Cell Carcinoma of the Bladder

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Background: It has been shown that expression and activity of lysosomal proteolytic enzymes (i.e., cathepsin B) correlate with tumor progression in various neoplasms. We investigate possible correlation of cathepsin B concentrations with grading and invasivity of tumorous bladder tissue. *Method:* Cathepsin B concentrations in serum and urine were measured in 40 patients (29 men, 11 women, mean age 68 years) with transitional cell carcinoma (TCC) of the bladder without metastases and in control group of 64 healthy subjects (28 men, 36 women, mean age 55 years) using commercially available enzymatic immunoassay. Concentration of cathepsin B in urine was adjusted on creatinine. Urinary creatinine in all samples was measured by enzymatic creatinase method. Patients were divided into groups according to the grading (low grading: 18 patients, high grading: 22 patients) and invasivity of the carcinoma (nonmuscle-invasive tumors: 23 patients, invasive tumors: 17 patients). *Result:* Concentrations of cathepsin B in urine were significantly elevated in patients than in control group (Median = 3.87 μg/L vs. 1.35 μg/L, $P = 0.0002$). Similarly, the ratio of U-cathepsin B/creatinine was significantly higher in patients (Median: 0.44 μg/mmol creatinine vs. 0.17 μg/mmol creatinine, $P < 0.0001$). U-cathepsin B may prove to be useful biomarker (area under the curve [AUC] = 0.72 and 0.73 for the U-cathepsin B/creatinine ratio, respectively). S-cathepsin B significantly correlated with grading of carcinoma ($P = 0.02$) and U-cathepsin B and U-cathepsin B/creatinine are positively associated with invasive tumors ($P = 0.0001$ and $P = 0.002$). *Conclusion:* Cathepsin B concentrations correlate well with grading and invasivity of tumors and may have diagnostic value in investigation of bladder cell carcinoma. New index U-cathepsin B/Creatinine ratio is more appropriate biomarker to monitor TCC, than U-cathepsin B so far. J. Clin. Lab. Anal. 26:61–65, 2012.

Key words: transitional cell carcinoma; bladder; cathepsin B; invasive; noninvasive tumors

INTRODUCTION

Cathepsin B is a papain-family cysteine protease synthesized as glycosylated preprotein of 37–49 k (pro-cathepsin B) and subsequently converted to active form of 33 kD single chain after the proteolysis of signal sequence (1). Cathepsin B is normally located in lysosomes, where it is involved in the turnover of proteins and plays various roles in maintaining the normal metabolism of cells (2). This protease has been implicated in pathological conditions, for example, tumor progression and arthritis. Imbalances between proteinases and their inhibitors correlate with tumor progression (3). In disease conditions, increases in the expression of cathepsin B occur at both gene and protein levels. At the gene level, the altered expression results from gene amplification, elevated transcription, use of alternative promoters, and alternative splicing. These molecular

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changes lead to increased cathepsin B protein levels and in turn redistribution, secretion, and increased activity (4).

Urothelial carcinomas, transitional cell carcinoma (TCC), are the fifth most common tumors after prostate cancer, breast cancer, lung cancer, and colorectal cancer (5, 6). Bladder carcinoma is the most common malignancy of the urinary tract. TCC comprises approximately 90% of primary bladder tumors. The other 10% are squamous cell carcinoma, adenocarcinoma, sarcoma and small cell carcinoma. TCC is characterized as malignant epithelial neoplasm of the urothelium with transitional cell differentiation, usually papillary character. There is highest occurrence of TCC in the bladder, less often it occurs in the ureter and in the renal pelvis. At the initial diagnosis of bladder carcinoma, approximately 70% of patients with bladder carcinoma are diagnosed with disease confined to the mucosa or submucosa (superficial stages Ta, Cis, or T1) and approximately 30% show muscle-invasive disease (7). Recurrence rate of patients with nonmuscle-invasive bladder carcinoma is 60–70%. Approximately 20–30% of patients with nonmuscle-invasive tumors subsequently develop invasive or metastatic cancer (8). The most useful prognostic parameters for tumor recurrence and subsequent malignant progression in the patient with nonmuscle-invasive tumors are the tumor grade, the depth of tumor penetration (stage), and the presence of carcinoma in situ. Thus the identification of markers that can be involved in bladder tumor progression is important. Only limited data have been published on bladder TCC and concentration or enzymatic activity of cysteine proteases including cathepsin B (9). We investigate possible diagnostic value of urine and serum cathepsin B concentrations and their correlation with grading and invasivity in TCC of the bladder.

PATIENTS AND METHODS

Urine and serum samples of patients with bladder cancer were obtained in the morning before surgical removal of their bladder tumor. Cancer diagnosis was detected by histological examination of tumor specimens obtained by transurethral resection or cystectomy.

These urine and serum samples were frozen immediately and kept at –70°C until cathepsin B was analyzed. Urine and serum concentrations of cathepsin B were measured in 40 patients (29 men and 11 women, mean age 68 years) with TCC of the bladder, and control group consisted of 64 healthy individuals (28 men, 36 women, mean age 55 years). Serum and urine levels of cathepsin B were assayed using commercially available immunoassay technique (EIA, Quantikine Assay, RD Biosystems, Abingdon, UK). Urine cathepsin B concentrations were adjusted to creatinine measured by enzymatic colorimetric creatinase method on the Advia 1800 analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY). Thus, urine cathepsin B concentration was expressed as μg of cathepsin B/mmol creatinine. Creatinine was measured in collected urine.

Grading and invasivity of the tumor were investigated by common histopathologic techniques. Patients were divided into groups—low-grade tumors or high-grade tumors—according to the grading of malignity by the WHO system (10) and depth of invasivity of the carcinoma—nonmuscle invasive (pTa and pT1) and invasive (pT2, pT3, and pT4) (11).

Characteristics of the patients and healthy subjects are summarized in Tables 1–3. Reference values for cathepsin B in serum and urine were adapted from the ELISA kit supplier. The reference values for S-cathepsin B were in the range of 27–126 μg/L, the reference values of U-cathepsin B were in the range of 0–2.54 μg/L.

Statistical Analysis

Differences between subgroups were tested for statistical significance by the nonparametric Mann–Whitney *U*-test. Value of $P < 0.05$ was considered as statistically significant. GraphPad Prism version 5.03 (GraphPad Software, San Diego, CA) was used for statistical analysis. Receiving operation analysis (ROC) was used to investigate the value of AUC. Statistical software GraphPad Prism, version 5.0 (San Diego, CA) and CB Stat, version 3.7 (Kristian Linnet, DK) were used to perform the statistical analysis.

TABLE 1. Characteristics of Patients and Healthy Subjects

	Control group ($N = 64$)	Patients ($N = 40$)	*P*-value
Men/women	28/36	29/11	—
Mean age (range)	55 (22–85)	68 (26–91)	—
S-cathepsin B (μg/L) median (range)	50.18 (4.3–102.4)	54.20 (22.60–184.7)	0.07
U-cathepsin B (μg/L) median (range)	1.35 (0.03–35.31)	3.87 (0.03–33.1)	0.0002
U-creatinine (mmol/L) median (range)	8.84 (2.29–24.21)	10.02 (0.97–25.91)	0.82
U-cat B/U-crea (μg/mmol creatinine) median (range)	0.17 (0–2.77)	0.44 (0–2.48)	<0.0001

Note: P-value is calculated from Mann–Whitney test.
U-cat B, urinary cathepsin B; U-crea, urinary creatinine.

TABLE 2. Characteristics of Patients According to the Grading of the Carcinoma

	Low grade TCC ($N = 18$)	High grade TCC ($N = 22$)	P-value
Men/women	13/5	16/6	—
Mean age (range)	69.5 (48–91)	66 (26–87)	—
S-cathepsin B (μg/L) median (range)	49.30 (22.60–136.0)	69.90 (23.60–184.7)	0.02
U-cathepsin B (μg/L) median (range)	2.96 (0.14–20.0)	5.28 (0.03–33.1)	0.18
U-creatinine (mmol/L) median (range)	10.78 (1.40–21.78)	6.60 (0.97–25.91)	0.61
U-cat B/U-crea (μg/mmol creatinine) median (range)	0.26 (0.01–1.50)	0.61 (0.005–2.48)	0.06

Note: P-value is calculated from Mann–Whitney test
U-cat B, urinary cathepsin B; U-crea, urinary creatinine; TCC, transitional cell carcinoma.

TABLE 3. Characteristics of Patients According to the Invasivity of the Carcinoma

	Nonmuscle-invasive tumors ($N = 23$)	Muscle-invasive tumors ($N = 17$)	P-value
Men/women	16/7	13/4	—
Mean age (range)	71 (26–91)	65 (46–86)	—
S-cathepsin B (μg/L) median (range)	50.7 (23.5–184.7)	65.1 (22.6–145.4)	0.18
U-cathepsin B (μg/L) median (range)	1.98 (0.03–9.90)	6.24 (1.53–33.07)	0.0001
U-Creatinine (mmol/L) median (range)	7.49 (0.97–18.75)	11.8 (1.57–25.91)	0.19
U-cat B/U-crea (μg/mmol creatinine) median (range)	0.27 (0.005–2.48)	0.92 (0.13–2.00)	0.002

Note: P-value is calculated from Mann–Whitney test.
U-cat B, urinary cathepsin B; U-crea, urinary creatinine; TCC, transitional cell carcinoma.

RESULTS

Urine levels of cathepsin B in patients with transitional cell bladder carcinoma were significantly higher than in the control group ($P = 0.0002$ for urine cathepsin B and $P < 0.0001$ for the ratio of urine cathepsin B/creatinine respectively) (see Table 1).

Serum levels of cathepsin B in patients with high-grade carcinoma are significantly elevated ($P = 0.02$). Urine levels of cathepsin B and ratio of urine cathepsin B/creatinine are not significantly different in patients with low-grade and high-grade carcinoma ($P = 0.18$ and $P = 0.06$ respectively) (see Table2).

Urine levels of cathepsin B and ratio of urine cathepsin B/creatinine are significantly elevated in patients with muscle-invasive tumors ($P = 0.0001$ and $P = 0.002$). Serum levels of cathepsin B in patients with muscle-invasive tumors are not significantly different from levels in patients with superficial tumors ($P = 0.18$) (see Table 3).

Diagnostic efficiency for cathepsin B ranges from AUC = 0.61 for serum cathepsin B to AUC = 0.73 for urine cathepsin B/creatinine, thus, urine cathepsin B concentrations and the ratio of urine cathepsin B/creatinine show good diagnostic efficiency in TCC of the bladder (see Table 4).

DISCUSSION

Results of this study confirm the role of cathepsin B as a potential tumor marker in investigation of the bladder TCC. Cathepsin B in serum and urine are elevated in comparison with the control group. These findings correlate with results of other studies investigating activities and concentrations of cathepsins in TCC (9, 12–15). Cathepsin B is molecular protein (33 kDa) secreted in urine in most malignant processes. Thus, concentration of cathepsin B in urine can be elevated due to the glomerular filtration. Concentrations of cathepsin B in urine depend on diuresis. Correction of urine cathepsin B concentration to the creatinine is needed to eliminate the impact of the diuresis.

Serum cathepsin B concentrations show significant positive correlation with grading of the TCC ($P = 0.02$), although urinary cathepsin B concentrations and cathepsin B/creatinine ratio are not significant ($P = 0.18$ and 0.06). This finding could be supported by the hypothesis that invasive high-grade bladder carcinomas are often associated with lymph node metastase in pelvic chains (25% of all invasive tumors) (16), with distant metastase including lung, liver, bone, neck, and central nervous system (17–19) and thus show high degree of angioinvasivity. High degree of angioinvasivity leads to the elevated transfer of cathepsin B through vessel wall and increased concentration in serum. Elimination of cathepsin B from serum is significantly decreased than in urine. This hypothesis is supported by the fact that in the group of 22 patients with HG tumors, 17 patients have invasive tumours and five of 17 patients with invasive tumors show metastases. From the results presented in Table 2 it is evident, that correction of the urine cathepsin B to the creatinine improves their significance (from $P = 0.18$ to $P = 0.06$) in comparison of TCC grading.

TABLE 4. Diagnostic Efficiency of Cathepsin B

	AUC (95% CI)	Cut-off (μg/L)	Specificity (%)	Sensitivity(%)	PPV (%)	NPV (%)
S-cathepsin B (μg/L)	0.61 (0.49–0.72)	73.5	92	33	83	68
U-cathepsin B (μg/L)	0.72 (0.61–0.83)	3.35	87	56	76	78
U-cat B/U-crea (μg/mmol creatinine)	0.73 (0.62–0.84)	0.33	89	59	77	78

Note: Cut-off values corresponding to maximal sensitivity and maximal specificity.
PPV, positive predictive value; NPV, negative predictive value.

The nonsignificant results for urinary cathepsin B correlate with results shown in other studies. The results of Staack et al. (9) ranging the *P*-value for urinary cathepsin B activities between 0.1 and 0.28 according to the grading of the TCC, similarly, the results of Svatek et al. (14) set the *P*-value for urinary cathepsin B activities at 0.43 and 0.557, respectively. Based on these findings and results of our study, we can suggest that serum cathepsin B is correlated just to the grading related to the grading of the TCC. It appears that the S-cathepsin B level correlates more with the size and surface of the tumors rather than invasivity.

Urinary cathepsin B levels correlate with invasivity of the tumor. Concentrations of urinary cathepsin B and ratio of U-cathepsin B/creatinine are significantly elevated in patients with muscle-invasive tumors ($P = 0.0001$ and $P = 0.002$). On the contrary, serum cathepsin B levels are not significant ($P = 0.18$). These results correlate with results of Eijan et al. (13), Staack et al. (15), Akpolat et al. (20), and Ueda et al. (21) demonstrating high expression of cathepsin B in TCC muscle-invasive tumors, on the contrary, results of Svatek et al. (14) did not show any significant tumor-related activity for cathepsin B.

Cathepsin B show diagnostic efficiency in TCC of the bladder ranging from poor (AUC = 0.61 for serum cathepsin B) to fair diagnostic efficiency (AUC = 0.72 and 0.73 for urinary cathepsin B and ratio of Urinary cathepsin B/creatinine, respectively). Despite the differences, statistical comparison of ROC curves show similar diagnostic efficiency of S-cathepsin B, U-cathepsin B and U-cathepsin B/creatinine ($P > 0.05$) as is shown in Figure 1.

Relationship of cathepsin B in patients will be viewed from several aspects: First, assessment against the population of healthy individuals is important for the diagnosis of primary tumor including screening and second, cathepsin B will be used to follow up examination and diagnosis of patients with tumor recurrence (mainly superficial tumors after transurethral resection) and relapse of disease in patients after cystectomy, thus reduction of necessary control cystoscopy would be assumed.

To assess the above-mentioned aspects of cathepsin B relationship in diagnostic process, clinically applicable cut-off value needs to be determined. The cut-off value for serum and urine cathepsin B was set at the value of maximal specificity and sensitivity and predictive values were

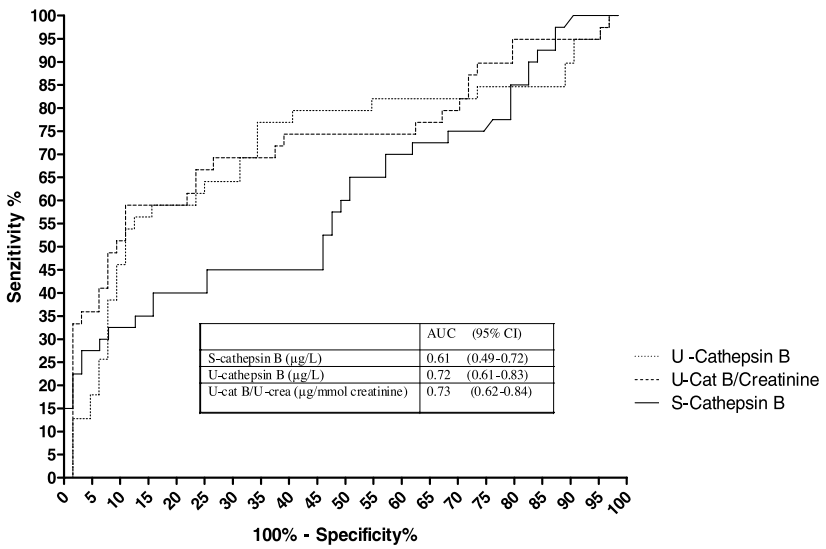

Fig. 1. Comparison of diagnostic efficiency of serum and urine cathepsin B.

calculated (see Table 4). Diagnostic specificity of cathepsin B ranged from 87% for U-cathepsin B with positive predictive value 76%, followed by the 89% for U-cathepsin B/creatinine with positive predictive value 77 and 92% for S-cathepsin B with positive predictive value 71%. This result confirms the importance of cathepsin B in diagnosis of bladder cell carcinoma and due to the low sensitivity ranging from 33 to 59% avoids the utility of cathepsin B in screening.

From the results it is well-known that investigation of the enzymatic concentrations correlates with the enzymatic activities reflecting gene expression and thus, measurement of cathepsin B concentration in serum and urine can be a potential tumor marker in patients with TCC of the bladder and may have important diagnostic value. New Index of U-cathepsin B/Creatinine ratio is more appropriate biomarker to monitor TCC, than cathepsin B used so far.

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